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THE PROTECTIVE EFFECT OF CINNAMON AGAINST MICRONUCLEI AND OXIDATIVE STRESS IN DIABETIC RATS

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ABSTRACT

This study was designed to evaluate the effect of cinnamon on the levels of micronuclei and antioxidant enzymes in alloxan-induced diabetic rats. Hyperglycemia was induced in rats by a single injection of alloxan at 150 mg/kg body weight intraperitonealy. Twenty four adult male rats were divided equally into four groups: Group I: control; Group II: diabetic rats; Group III: rats treated with cinnamon; Group IV diabetic rats treated with cinnamon. Rats were sacrificed after 2 weeks of cinnamon treatment and samples from bone marrow and liver prepared for the determination of micronucleus and antioxidant enzymes assays, respectively. Antioxidant enzymes such as superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA) were examined.

In rats bone marrow cells, no significant increases of the frequencies of cells with micronuclei were observed at dose of 150 mg alloxan/kg b. w. Combined treatment with alloxan and cinnamon failed to induce micronuclei in bone marrow cells.

In the present work, antioxidant parameters, superoxide dismutase and malondialdehyde were significantly increased in the liver tissue with a concomitant decrease in GSH in diabetic group. Treatment with cinnamon restored the activities of antioxidant enzymes of the diabetic rats. The present investigation suggest that cinnamon has a protective effect on the bone marrow and liver cells in experimental diabetes mellitus.

KEYWORDS: Alloxan (All), Cinnamon, Micronuclei (MN), Super Oxide Dismutase (SOD), Glutathione (GSH), Malondialdehyde (MDA)

INTRODUCTION

Diabetes is one of the components of metabolic syndrome regularly associated with cardiopathy, nephropathy, retinopathy and circulatory disease (Evangelista and McLaughlin, 2009, Lorenzi and Gerhardinger, 2001); decrease in the mRNA levels of liver fatty acid-binding protein, liver fatty acid-binding protein (L-FABP) (Fan et al, 2013). Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia that results from defects in insulin secretion, action, or both (Naziroglu et al, 2012). Diabetes mellitus is characterized by abnormalities in carbohydrate, lipid and protein metabolism due to complete or relative insufficiency of insulin secretion from pancreatic β-cells and/or defect in insulin action (Lin and Sun, 2010). It is one of the leading metabolic syndromes, accounts for highest morbidity and mortality worldwide (Najm and Lie, 2010).

The practice of a long-term chronic physical training protocol might be considered an important assistant in the treatment of diabetes, mitigating the occurrence of possible damages to liver tissue and control of changes in the lipid metabolism, in alloxan animals (Remedio et al, 2011 and Ribeiro et al, 2012).

On the other hand, Nymphaea stellata flowers extract did not caused genotoxic effects in Ames test, in the in vitro chromosomal aberration assay and in the in vivo micronucleus assay in alloxan diabetic rats (Huang et al, 2010). The frequencies of micronuclei and chromosomal aberrations had been significantly increased in diabetic mice (Farghaly and Hassan, 2012).

The cinnamon extract seemed to had a moderate effect in reducing fasting plasma glucose concentrations in diabetic patients with poor glycaemic control (Mang et al, 2006; Pham et al, 2007; Jia et al, 2009; Mishra et al, 2010). The alloxan-damaged pancreatic B-cells of the rats were partly recovered gradually after the rats were administered with cinnamon oil (CIO) carried by liquid-loadable tablets (CIO-LLTS) 45 days later as concluded by Han and Cui (2012). Studies have demonstrated many beneficial health effects of cinnamon, such as anti-inflammatory properties, anti- microbial activity, blood glucose control, reducing cardiovascular disease, boosting cognitive function, reducing risk of colonic cancer, attenuation of weight loss associated with diabetes; reduction of fasting blood glucose; reducing of low density lipoprotein (LDL)cholesterol and increasing high density lipoprotein (HDL) cholesterol; reducing glycosylated haemoglobin A1c (HbA 1C) and increasing circulating insulin levels (Subash Babu et al, 2007, Gruenwald et al, 2010). The present study sought to determine whether cinnamon would attenuate the micronucleated cells and antioxidant enzymes induced by intraperitoneal administration of alloxan in male rats.

MATERIALS AND METHODS

Test Agent

Alloxan (5, 6 Dioxyuracil) Monohydrate, C4H4N2O4. H2O FW 160.1 was purchased from Sigma Chemical Co. St. Louis MO 63178 USA. 314-771-5750. Cinnamon: Manufactured by Good N Natural, Bohemia, NY 11716 USA. All other chemicals used in the experiments were of analytical grade.

Animals: Male rats (100-150 gm) were divided into four groups of six animals each. Group 1 served as control and received distilled water only. Group 2 received cinnamon 100 mg/kg b.w. Group 3 received alloxan 150 mg/kg b.w. Group 4 received both cinnamon and alloxan.

Diabetes Induction and Drug Administration

Diabetes was induced in rats by a single intraperitoneal administration of 5% alloxan at 150 mg/kg body weight. This dose was determined according to Siddiqui et al. (2005). The blood glucose of the animals was tested after the third day of alloxan administration. After confirmation of induction using electronic Glucometer, rats with a fasting blood glucose (FBS) in the range of 175-300mg/DI were selected for study.

A dose of 1.5 ml of aqueous suspension of cinnamon/100 g b.w. (equivalent to 100 mg/100 g b.w.) was orally administered daily to alloxan-diabetic rats for 2 weeks. The dose of cinnamon was evaluated according to Jia et al.(2009). On day 14 of cinnamon treatment, the rats were killed by cervical dislocation under ether anesthesia.

Micronucleus Test

Animals were killed, both humeri of each animal were used for the micronucleus test (Adler et al, 1991). Bone marrow cells were flushed out with fetal calf serum, smeared, fixed with methanol, and stained with Giemsa. For each animal, 1000 PCEs were examined for the presence of micronuclei and expressed as micronucleated polychromatic erythrocytes (MNPCEs) per 1000 PCEs.

PCE/NCE ratios: An additional 1000 erythrocytes (PCES+ NCEs) were scored for PCE/NCE ratios in each group of rats (i.e. 6000 erythrocytes analyzed per group).

Antioxidant Enzymes

Liver tissue was removed from the samples to be used for determination of antioxidant enzymes.

Superoxide dismutase (SOD): The activity of superoxide dismutase was measured by the method of Sun et al. (1988). One unit of SOD activity was defined as the amount of enzyme that inhibited the rate of autoxidation of pyrogallol by 50%. Glutathione (GSH): Glutathione was determined according to the method of Beutler et al. (1963).

Malondialdehyde (MDA): Lipid peroxidation (malondialdehyde) was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA) a product formed due to the peroxidation of membrane (Ohkawa et al, 1979).

Statistical Analysis: The data were expressed as mean ± S.D. The results are analyzed statistically using one way ANOVA with Dunnett's tests (2-sided) as a post test. The minimum level of statistical significant was set at $P<0.05$.

RESULTS

Micronucleus Test

Control groups with distilled water treatment showed a range of 1-4 micronucleated cells per 1000 cells between individual rats. The incidence of MNPCEs in the bone marrow of rats treated with alloxan although slightly higher than the control was not significantly elevated (Table 1& Figure 1). Moreover, no significant difference could be found in the frequency of micronucleated cells among the other treated groups. The number of cells containing multiple MN did not differ among the groups. Moreover, as shown in Table 1 there was very little effect of cinnamon on micronucleated cells produced by alloxan. Pretreatment with cinnamon alone had no effect on micronuclei. The frequencies of %N observed in alloxan treated groups were significantly different from control values. The ratio of polychromatic erythrocytes to normochromatic erythrocytes ranged between 1.06-0.75. Also, a significant drop in both PCEs and PCEs/NCEs ratios were observed in the bone marrow of rats treated with alloxan.

Antioxidant Enzymes

In diabetic animals, a significantly ($p\leq0.001$) increased activity of superoxide dismutase was observed in comparison with healthy controls. Treatment with cinnamon in diabetic rats resulted in reversal of SOD activity to values still slightly above normal. GSH activity in liver in untreated diabetes rats was significantly reduced compared with healthy controls, whereas treatment with cinnamon for 14 days restored it to near-normal values in the respective treatment groups.

The lipid peroxide in diabetic rats was increased significantly $p\leq0.001$. The levels were altered to near normal in cinnamon treated alloxan induced diabetic rats (Table 2). Discussion Diabetes is a chronic metabolic disorder in humans constituting a major health concern today whose prevalence has continuously increased worldwide over the past few decades (Lachin, 2014). It is a common disorder associated with increased morbidity, mortality rate, and can be characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both, causing metabolic and physiological changes in various organs (Genet et al, 2002). Also, Diabetes mellitus is a chronic metabolic disorder that is associated with an increased risk of cardiovascular disease, retinopathy, nephropathy, neuropathy, sexual dysfunction and periodontal disease. Improvements in glycaemic control may help to reduce the risk of these complications (Leach and Kumar, 2012).

Persistent hyperglycemia causes increased production of free radicals in all tissues, especially reactive oxygen species (ROS) from glucose auto-oxidation and protein glycosylation (Naziroglu and Butterworth, 2005). Reactive oxygen species (ROS)-induced pancreatic β-cell death has an important role in the pathogenesis of diabetes and also affects insulin secretion. The ROS that are particularly responsible for oxidative stress include superoxide ions (O2-), hydroxyl radicals (OH), singlet oxygen (1O2), hydrogen peroxide (H2O2), nitric oxide (NO) and peroxynitrite (ONOO-). Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids, lysosomes, nucleic acid and eventually lead to cell death,events related to late diabetic complications (Evans et al, 2003&Maritim et al, 2003 and Lachin, 2014).

The diabetogenic agent alloxan is a hydrophilic and chemically unstable pyrimidine derivative, which is toxic to pancreatic β-cells because it can generate toxic-free oxygen radicals during redox cycling in the presence of reducing agents such as glutathione and cysteine (Szkudelski, 2001).

This drug induces diabetes by intracellular generation of ROS formed in a cyclic reaction involving alloxan and its reduced product called dialuric acid, with subsequent inhibition of insulin synthesis and secretion (Behr et al, 2008). A low dose of alloxan (120 mg/kg) causes partial destruction of pancreatic β-cells in laboratory rodents and thereby produces glucose intolerance and hyperglycemia (Yadav et al, 2008).

There are many mechanisms by which the increased blood suger level is lowered in diabetic rats treated with an antioxidant agent. The increase in the glucose transport across cell membrane resulting in increased peripheral glucose utilization, increased glycogen synthesis from glucose, decreased glycogenolysis in liver, increased insulin release from beta cells of islet of Langerhans in pancreas, increase sensitivity of insulin receptors, decreased insulin resistance, and decreased glucose absorption from intestine (Sachdev et al, 2012).

The rodent erythrocyte micronucleus (MN) assay in peripheral blood or bone marrow is considered to be the primary assay to assess in vivo genotoxic potential (Eastmond et al, 2009). Micronuclei (MN) are surrogate measures of structural and numerical chromosomal aberrations that are associated with increased cancer risk (Bonassi et al, 2007). MN can also be considered bridging biomarkers of genotoxic exposure, since they can be enumerated across multiple species including humans (Dertinger et al, 2007).

This investigation was undertaken to evaluate the role of cinnamon in an alloxan-induced diabetic model and the oral glucose tolerance test in rats. The results obtained in MN tests clearly demonstrated that alloxan was not genotoxic at the dose studied, as the frequencies of MNPCEs in rats treated with alloxan did not differ significantly from those of the control. Since the results indicated that treatment did not result in the production of significant increases in micronucleus frequency in male rats, alloxan was not considered to be clastogenic in this assay system. Treatment with cinnamon at 100mg/kg b.w. for 14 days failed to induce micronuclei in bone marrow cells. Simultaneous treatment with alloxan and cinnamon did not produce appreciable increases in micronuclei or micronucleated cells over background or their respective controls. Cinnamon-induced adaptive response demonstrated in the present study to alloxan could therefore possibly be attributed to the involvement of antioxidant defence system. To the author’s opinion, two reasons may be given for the noninduction of MN in bone marrow cells: 1-The drug metabolites reaching the bone marrow were not sufficient to induce MN. 2-The MNPCEs formed at an early phase of bone marrow erythropoiesis might have entered the circulation at the time of sampling. Major factors contributing to the sensitivity of the bone marrow MN test are cellular kinetics and DNA repair capacity of the target organ and pharmacodynamics of the test compound.

However, the mean relative number of NCEs, was significantly increased after treatment with alloxan as compared to the mean values of NCEs of the corresponding controls, indicating that alloxan had cytotoxicity properties on the bone marrow cells as suggested by Jenssen and Ramel (1978). This shift is attributable to the influx of peripheral blood to fill up the medullary space following bone marrow depletion. Similarly, the percentage of PCEs value dropped significantly in rats treated with alloxan. Also, alloxan inhibited cell proliferation which is expressed as a decline in the PCEs/NCEs ratio. There was a significant decline in PCEs/NCEs ratio after treatment with alloxan.

The micronucleus test is a comparatively rapid and sensitive indicator of both chromosome aberrations and the chromosomal losses that lead to numerical chromosome aberrations. It can detect clastogens and spindle poisons (Heddle et al, 1983). To the author's opinion, it believe that micronuclei formed with alloxan induction arise from acentric chromatid fragments instead of total chromosomes. These results indicate that this drug is effective in the S and G2 phase of cell cycle. The appearance of more than one micronucleus in PCEs was related to the results of chromosome aberrations.

A cytogenetic study of bone marrow cells was carried out under conditions of alloxan diabetes in rats. These experimental models might be used in research on medicinal mutagenesis (Zolotareva et al, 1984). Moreover, zinc-insulin therapy failed to compensate the diabetes completely, but normalized to a considerable measure the diminished proliferative activity of the lymphoid cells of the thymus and bone marrow of male mice with alloxan diabetes (Kozlov and Loktiushina, 1992). It has been hypothesized that increased ROS generation in long-standing diabetes might result in oxidative damage to DNA (Shanthi and Ramakrishnan, 1994 and Wyatt et al, 2006). Alloxan induced DNA cleavage frequently at thymine and cytosine residues in the presence of NADH and Cu (II) (Murata et al, 1998). Simillarly, it induced DNA damage in normal human lymphocytes in a dose-dependent manner using the alkaline comet assay (Blasiak et al, 2003).

The extract from Nymphaea stellata flowers exhibited significant intestinal alpha-glucosidase inhibitory activity, without showing any acute toxicity or genotoxicity, which might be useful in suppressing postprandial hyperglycemia in diabetics (Huang et al, 2010). An elevation of all biomarkers for oxidative stress, generation of reactive oxygen species and activation of apoptotic signal proteins like NFkB and down regulation in expression of marker proteins like insulin, GLUT2 and glucokinase and change in structure and conformation of DNA in hyperglycemic mice were observed by Samadder et al. (2011).

The present result is not in accordance with the results of (Farghaly and Hassan, 2012) who mentioned thatalloxan caused an increase in the frequencies of micronuclei and chromosomal aberrations in mice compared with the control. Furthermore, they showed that Lupinus termis methanolic extract (LTE) was a suitable agent for preventing diabetes mellitus-induced DNA damage.

On the other hand, major antioxidant enzymes, such as SOD, glutathione and malondialdehyde were studied to evaluate the antioxidant status in liver tissue of cinnamon-treated diabetic rats.

As mentioned in the present study, diabetic rats exhibited an increase in the levels of SOD. This was accordance with the results obtained by Sabu and Kuttan (2009) who showed increase in superoxide dismutase in blood and liver of diabetic rats. The present work showed decreased level of glutathione (GSH) in liver of alloxan induced rats. This was accordance with the results obtained by Prince and Srinivasan (2005), Kaimal et al. (2010), Sharma and Garg (2012) who noticed significant decreased in glutathione (GSH) content in liver, kidney and pancreas of diabetic rats.

In addition, MDA formation, the index of lipid peroxidation, was significantly increased in the liver tissue of ALX-treated animals. Elevation of (lipid peroxides) LPO was attributed to the enhanced production of the reactive oxygen species.

This was accordance with the results obtained by Kaimal et al. (2010) who noticed significant increase in lipid peroxides in liver and pancreas of alloxan induced diabetic rats.

In addition, increased oxidation of free fatty acids (FFA) by the liver can generate ROS, which can lead to lipid peroxidation processes, structural and functional alterations in cells and cell death (Haque and Sanyal, 2002). Ananthan et al. (2004) concluded that, in diabetes, liver and kidney tissues were more vulnerable to oxidative stress and showed increased lipid peroxidation.

During oxidative stress, there was an overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) on one side, and a deficiency of enzymatic and nonenzymatic antioxidant defense system on the other, resulting in degradation of cellular components, DNA, carbohydrates, proteins and lipids (Halliwell and John, 1989). Multiple effects of alloxan on functional integrity and antioxidant enzyme gene expression in clonal beta-cells was indicated by Picton et al. (2002).

Cinnamon supplementation potentially increased GSH level, with subsequent decreased levels of SOD and MDA, suggesting that cinnamon might have antioxidant principles to produced such a response.

Similarly, this was accordance with the results obtained by (Ghosh et al, 2011, Middha et al. 2011, Sharma and Garg, 2009, Farombi and Ige, 2007, Prince et al, 2004, Satheesh and Pari, 2004, and Yang et al, 2002). They found that administration ofbacosine and glibenclamide, Trigonella foenumgraceum, Butea monosperma, Hibiscus sabdariffa, Tinospora cordifolia, Boerhavia diffusa and Pedicalaris decormsignificantly decreased levels of SOD, MDA and/or increased the levels of reduced glutathione (GSH) in the hepatic, pancreatic and renal tissues of diabetic rats.

The anti oxidative effect of cinnamon was monitored under the experimental conditions of alloxan-induced diabetes mellitus. The present result is agreement with the results obtained by Leach and Kumar (2012) who found cinnamon to be no more effective than placebo, another active medication or no treatment in reducing glucose levels and glycosylated haemoglobin A1c (HbA1c), a long-term measurement of glucose control. Cinnamomum zeylanicum demonstrated numerous beneficial effects both in vitro and in vivo as a potential therapeutic agent for diabetes (Ranasinghe et al, 2012). The mode of action for this hypoglycaemic action is unclear, but may be attributed to an increase in serum insulin levels, hepatic glycogen storage (Subash Babu et al, 2007), improved insulin-receptor signalling (Qin et al, 2004), an insulinomimetic effect (Roffey et al, 2006), or a reduction in intestinal alpha-glucosidase activity (Kim et al, 2006).

Finally, cinnamon pretreatment at 100mg/kg b.w. concentrations slightly reduced the percentage of micronucleated cells and increased of glutathione accompanied with subsequent decreased content of both superoxide dismutase and malondialdehyde induced by 150 mg/kg of alloxan. To the author′s opinion, the antioxidant responsiveness mediated by cinnamon might be anticipated to have biological significance in eliminating reactive free radicals that might otherwise affect normal cell functioning and provide a scientific rationale for the use of cinnamon as an antidiabetic plant. The findings of the present study shows positive effects of cinnamon on rats with ALX-induced disturbances in the micronucleated cells levels and the antioxidants status. Thus, cinnamon is beneficial in the control of diabetes and oxidative stress by activation of enzymatic and non-enzymatic antioxidants. Numerous reports together with the present

results have proved the efficacy of antioxidative supplements administration in the prevention of diabetic complications. Further trails investigating long-term benefits and risks of the use of cinnamon for diabetes mellitus are required.

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APPENDICES

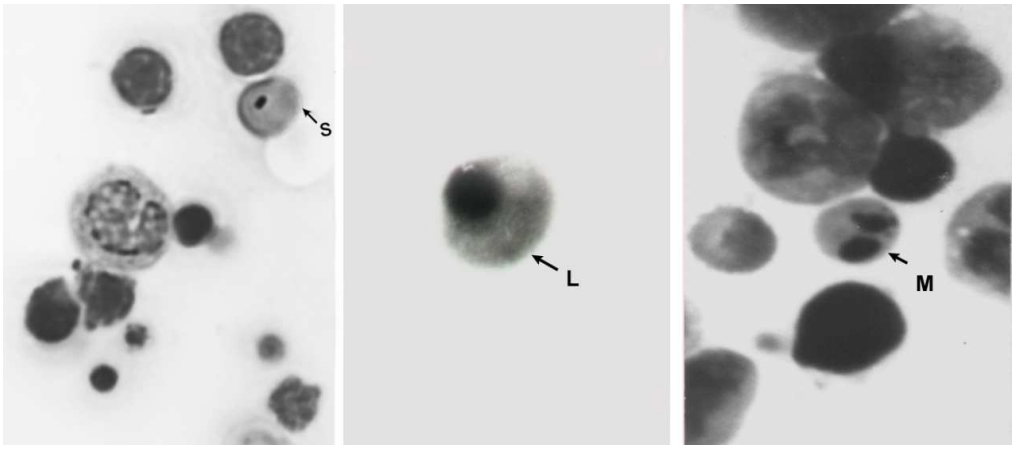

Figure 1: Various Shapes of Micronucleated Polychromatic Erythrocytes in Bone Marrow from Male Rats Treated with a Single Intraperitoneal Injection of Alloxan and/or Cinnamon S=Small Micronucleus L=Large Micronucleus M=More than One Micronuclei

Table 1: Frequencies of Bone Marrow Micronuclei Induced by Alloxan and/or Cinnamon in Male Rats

Experimental Group	Number of Examined Cells	Total MN	Mean ± S. D	MN %	PCEs No. PCEs%	NCEs No. NCEs%	PCEs/ NCEs Ratio
Control	6000	13	2.16±0.40	0.21	3001 50.01	2999 49.98	1.0006
Cinnamon	6000	15	2.5±1.37	0.25	3091 51.51	2909 48.48	1.06
Alloxan	6000	22	3.66±2.94	0.36	2581 43.01***	3419 56.98***	0.75***
Alloxan + Cinnamon	6000	17	2.83±1.16	0.28	2931 48.85	3069 51.15	0.95

PCEs=polychromatic erythrocytes NCEs= Normochromatic erythrocytes

PCEs/NCEs ratio = The ratio of PCEs to NCEs ***= alloxan significantly different from control ($p<0.001$)

Table 2: The Effect of Alloxan and/or Cinnamon on Superoxide Dismutase,

Glutathione and Malondialdehyde in Liver of Male Rats

Group / Parameters	Control Mean±SD	Cinnamon Mean±SD	Alloxan Mean±SD	Alloxan + Cinnamon Mean±SD
Superoxide dismutase (SOD)	6.73±1.88	6.63±0.59	18.63±2.02***	8.41±0.97-
Glutathione (GSH)	2.68±0.76	3.65±0.98*	0.37±0.11***	1.60±0.47**
Malondialdehyde (MDA)	0.82±0.07	0.32±0.08*	3.83±0.67***	0.99±0.13-

- = $p \geq 0.05$ non significant *= $p \leq 0.05$ significant **= $p \leq 0.02$ highly significant

***= $p \leq 0.001$ very highly significant